Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Virology 446 (2013) 123–132

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Review

Non-encapsidation activities of the capsid proteins of positive-strand RNA viruses

Peng Ni, C. Cheng Kao *

Department of Molecular & Cellular Biochemistry, Indiana University, Bloomington, IN 47405, USA

ARTICLE INFO

Article history:
Received 26 March 2013
Returned to author for revisions
11 July 2013
Accepted 20 July 2013
Available online 27 August 2013

Keywords:
Capsid protein
Positive-strand RNA virus
Protein–RNA interaction
Protein–protein interaction
Regulation of viral infection
Review

ABSTRACT

Viral capsid proteins (CPs) are characterized by their role in forming protective shells around viral genomes. However, CPs have additional and important roles in the virus infection cycles and in the cellular responses to infection. These activities involve CP binding to RNAs in both sequence-specific and nonspecific manners as well as association with other proteins. This review focuses on CPs of both plant and animal-infecting viruses with positive-strand RNA genomes. We summarize the structural features of CPs and describe their modulatory roles in viral translation, RNA-dependent RNA synthesis, and host defense responses.

Contents

Introduction

Positive (+)-strand RNA viruses comprise more than a third of all virus genera. The genomes of (+)-strand RNA viruses are also

* Corresponding author.
E-mail address: ckao@indiana.edu (C. Cheng Kao).

0042-6822/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.virol.2013.07.023

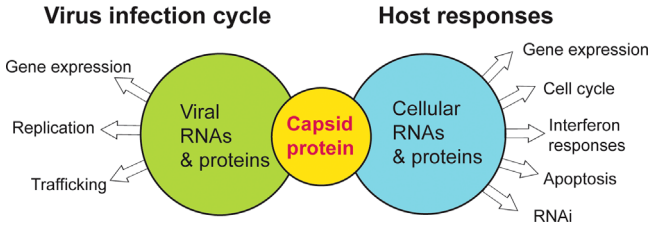

Fig. 1. Potential regulatory activities of viral CPs.

among the smallest, with a typical coding capacity being less than a dozen proteins. The economy of the genome does not obviate the need to overcome host defenses as well as to coordinate the production of viral molecules. Therefore, many viral proteins have multiple functions, including the structural proteins.

The viral capsid protein (CP) is a structural protein that accompanies the viral genome into and out of cells. CPs are usually among the most abundant viral proteins made during infection. In addition to mediating entry and forming the protective shell for the viral genome, CPs can regulate viral gene expression, RNA synthesis and virus-host interactions (Fig. 1).

This review focuses on the non-traditional roles of the CPs of spherical (+)-strand RNA viruses. Other commonly used names are coat protein (CP), core protein and nucleocapsid protein. This review is not intended to be an exhaustive survey of all the reported regulatory functions of CPs, but to illustrate themes in animal and plant viruses with (+)-strand RNA genomes and to provide specific examples. Our review is an extension of a number of previous reviews on this subject and we encourage interested readers to seek them out (Bol, 2008; Callaway et al., 2001; Urbanowski et al., 2008).

Structural features of CP

The function of the CP, including those that are not directly related to viral genome encapsidation, can best be considered in the context of the CP structure. CPs of spherical (+)-strand RNA viruses lack sequence homology and vary in size across genera. However, several common structural features of the CPs can be recognized (Rossmann and Johnson, 1989).

The CPs of spherical (+)-strand RNA viruses are generally single gene products, except for members in families such as *Secoviridae* and *Picornaviridae*. With some exceptions, the CP monomers contain an extended and highly flexible N-terminal arm (NTA) followed by a more structured region(s). The structured region contains a domain that serves to encase the viral RNA, commonly called the shell (S) domain, and in most cases, a short C-terminal extension. Alternatively, in taxa such as members of the *Tombusviridae* and *Caliciviridae*, the S domain is followed by one or more protruding (P) domain(s) that project outside the capsid (Rossmann and Johnson, 1989).

Structurally flexible regions in the CP

A significant portion of the CPs of both nonenveloped and enveloped spherical (+)-strand RNA viruses are structurally flexible. In addition to being difficult to resolve in crystallographic structures, they are likely to be intrinsically disordered (Ivanyi-Nagy et al., 2008; Liljas, 2011). Intrinsically disordered sequences are typically characterized by a high proportion of polar and charged residues and underrepresentation of bulky hydrophobic residues. Furthermore, intrinsically disordered regions have high specificity but lower affinity in their interaction with ligands, a feature useful for multifunctional proteins (Dunker et al., 2008). Importantly, proteins with intrinsically disordered regions include ones that act as hubs to mediate a network of protein–protein interactions (Dunker et al., 2005). This feature likely applies to viral CPs.

The NTAs of the CPs of spherical (+)-RNA viruses is usually highly flexible (Fig. 2). They tend to be located within the internal cavity of the capsid and mediate interaction with RNA. Additionally, members of the *Bromoviridae*, *Tombusviridae*, *Nodaviridae*, *Togaviridae* and hepaciviruses of the *Flaviviridae* have NTAs enriched with positively-charged residues (Fig. 2: Boulant et al., 2005; Choi et al., 1991; Fisher and Johnson, 1993; Harrison et al., 1978; Lucas et al., 2002). Brome mosaic virus (BMV; *Bromoviridae*) with mutations in the charged residues of the NTAs exhibited both a change in the amount and the species of viral RNA encapsidated, indicating that the charged residues contribute to RNA binding by a combination of nonspecific electrostatic interactions and specific RNA recognition (Ni et al., 2012).

Additional functions of the NTAs include formation of an oligomerization network or switching the conformation of CP during assembly. This has been shown for the CP of the members of *Sobenomvirus*, *Tombusviridae*, *Nodaviridae and Caliciviridae* (Abad-Zapatero et al., 1980; Chen et al., 2006; Fisher and Johnson, 1993; Harrison et al., 1978; Prasad et al., 1999). With the nonenveloped viruses, the NTA may function in virion trafficking in the cell and/or RNA release. The NTAs of Cowpea chlorotic mottle virus (CCMV: *Bromoviridae*) and Cucumber necrosis virus (CNV; *Tombusviridae*) are highly sensitive to proteases, suggesting that they can extend outside of the virion (Kakani et al., 2004; Speir et al., 2006). Intriguingly, the NTA of the CNV CP facilitates interaction with cellular filaments to mediate virion trafficking to plastids (Xiang et al., 2006). These reports suggest that flexibility of the CP arms to shuttle from the interior to the exterior of the capsid are involved in the virion's translocation.

Structured domains of CPs

The structured portion of the CPs displays several distinct folds. The most prevalent is the “jelly-roll” fold found in almost all the S domains of the CPs of nonenveloped spherical (+)-strand RNA viruses (Rossmann and Johnson, 1989). It consists of two back-to-back four-stranded β-sheets (Fig. 2A) and form the icosahedral shell with contributions from the extended N- and C-terminal portion of the CP. Notably, there is high variability in the structure of the loops that connect the β-strands of the jelly roll fold.

In the cases of *Tombusviridae* and *Caliciviridae*, the P domain that follows the S domain folds into β-barrel conformations (Fig. 2A; Harrison et al., 1978; Prasad et al., 1999). The P domains contain binding sites for receptors and undergoes genetic changes more rapidly in comparison to the S domain (Donaldson et al., 2010; Katpally et al., 2010). The P domain also participates in the dimeric interaction of CP subunits and influences capsid stability (Bertolotti-ciarlet et al., 2002). Additionally, the hinges between the P and S domains or between the subdomains within the P domain are highly flexible, allowing the P domain to rotate for dimerization or for interaction with receptors (Kakani et al., 2008).

The CP of *Leviviridae* bacteriophages, such as MS2 has a unique fold among nonenveloped spherical (+)-strand RNA viruses. The MS2 CP is entirely ordered and forms a rigid dimer consisting of two long α-helices juxtaposed on a flat β-sheet (Fig. 2B; Valegård et al., 1990). In the absence of the terminal arms, the β-sheet is responsible for interaction with the viral RNA (Valegård et al., 1994).

The structural domains of enveloped (+)-strand RNA viruses do not share a common fold. For instance, the C-terminal portion of the CP of Sindbis virus (SINV; *alphaviridae*) adopts a chymotrypsin-like protease topology and is arranged with T=4 symmetry in the nucleocapsid core (Fig. 2B, Choi et al., 1991). The central region of

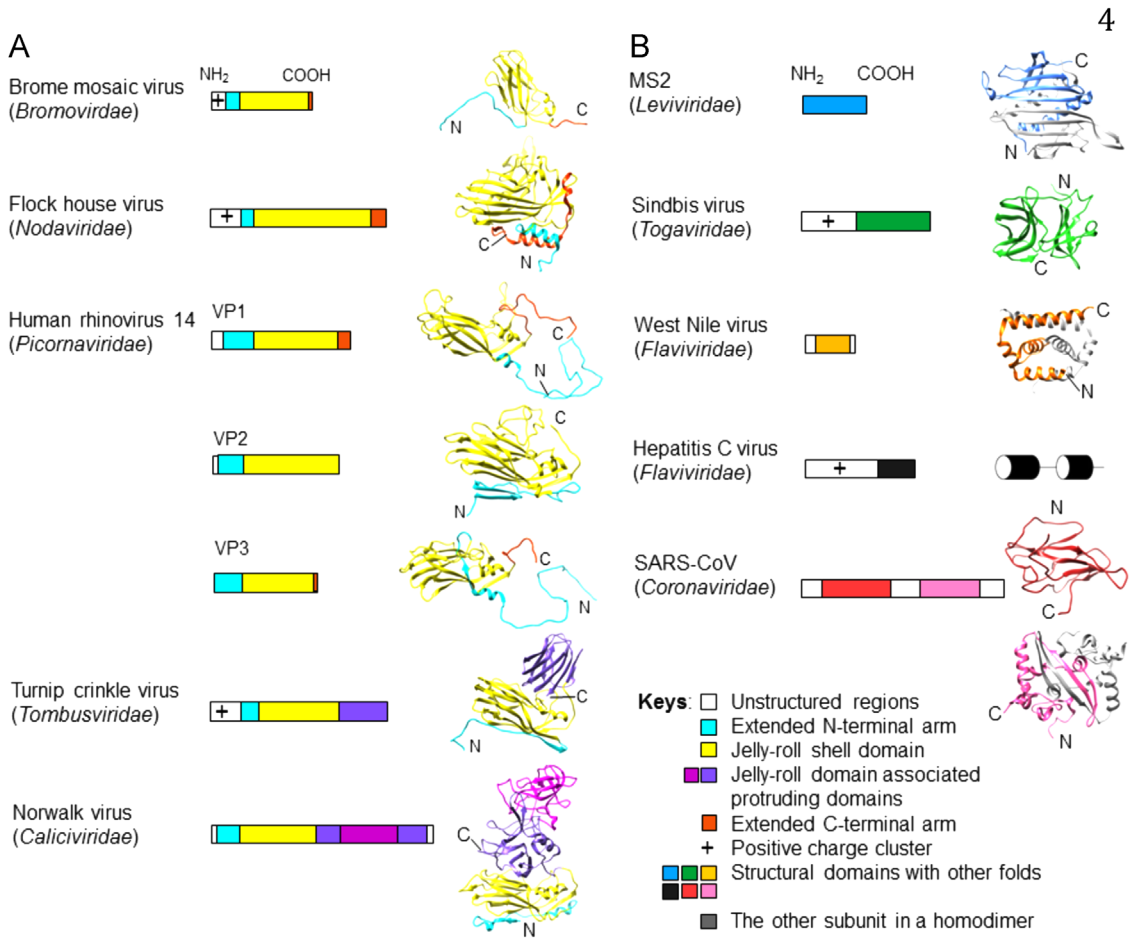

Fig. 2. Schematics and structures of representative CPs of spherical (+)-strand RNA viruses. (A) CPs with jelly roll shell domain and associated motifs. This category includes majority non-enveloped spherical (+)-strand RNA viruses. Specific examples used to illustrate the variations in the CPs include the B subunit of an asymmetric trimer of Brome mosaic virus CP (PDB: 1js9), the B subunit of Flockhouse virus CP (PDB: 4ftb), the VP1, VP2 and VP3 of human rhinovirus 14 (PDB: 4rhv), the C subunit of Turnip crinkle virus CP (PDB: 3zx8), the B subunit of Norwalk virus VP1 capsid protein (PDB: 1ihm). (B). The CPs of (+)-strand RNA viruses that use alternative structure to form the capsid. Viruses in this category include the non-enveloped *Leviviridae* and enveloped spherical (+)-strand RNA viruses. All CPs of enveloped spherical (+)-strand RNA viruses contain unstructured regions, which may also be enriched for positively charged residues. Examples used are the CP dimer of MS2 bacteriophage (PDB: 2ms2), the CP of Sindbis virus with chemotrypsin-like shell domain (PDB: 1kxa), the core protein of West Nile virus (PDB: 1sfk), the core protein of Hepatitis C virus (The cylinders represent putative α-helices), the nucleocapsid protein of SARS-Coronavirus (PDB: 1ssk and 2cjr for the N-and C-terminal structural domain respectively). A key to the structural elements used in the figures is in the lower right corner.

the core protein of the West Nile virus (WNV, *Flaviviridae*) dimerizes through α-helical bundles (Fig. 2B; (Dokland et al., 2004). The homodimer of WNV core proteins displays basic residues along one surface for RNA binding, and nonpolar resides on the other for membrane interaction.

The nucleocapsid (N) protein of the *Coronaviridae* is the only exception discussed in this review which wraps the RNA into a flexible filamentous ribonucleoprotein complex within the spherical envelop. The SARS coronavirus (SARS-CoV) N protein contains N-terminal and C-terminal structural domains that are flanked by highly flexible termini and a linker region (Fig. 2B). The N-terminal domain is known as the RNA binding domain and C-terminal domain as the dimerization domain (Chen et al., 2007; Huang et al., 2004; Takeda et al., 2008; Yu et al., 2006). However, the C-terminal dimerization domain and the three flexible regions are also involved in RNA binding (Chang et al., 2009).

Role of oligomerization

Regulatory activities of viral CPs can be affected by the amount of CP expressed. The CP concentration that is low in the initial phase of an infection typically increases dramatically near the end of one infection cycle. The increase in concentration drives the CP to assemble into higher-ordered structures, and the changes in CP oligomerization state serves as a regulatory switch to coordinate the progression of the infection process.

At the molecular level, the high-resolution structures of RNA-CP complexes involve dimer of CP or of CP peptides (Guogas et al., 2004; Valegård et al., 1994). Following addition of other dimers, the dimers of CPs may associate into higher-order intermediates, such as pentamers of dimers or trimers of dimers, which ultimately leads to the formation of capsid (Prasad et al., 1999; Rossmann et al., 1985; Sorger et al., 1986). Structures of a number of spherical (+)-strand RNA viruses have revealed the association of the RNA density with dimers, pentamers or hexamers of the CPs (Bottcher and Crowther, 1996; Chen et al., 1989; Fisher and Johnson, 1993), indicating that the RNA-CP interface may change with the oligomerization state of the CP. This, in turn, could influence the conformation and function of the RNA. The status of CP oligomerization also dictates its protein–protein interaction interface, as has been demonstrated for capsid assembly (Stockley et al., 2007). Although atomic structures of many capsids are well-characterized, the dynamic interaction between CP subunits or between CP and RNA is more difficult to capture. Nonetheless, it is likely that the oligomerization state of CPs significantly impact their regulatory activities.

Two activities form the basis for a number of regulatory activities of the viral CPs: (1) binding to RNA and (2) binding to other proteins. The propensity for CP to oligomerize in a concentration-dependent manner further modulates the regulatory activities of CPs. We cannot do full justice to the many systems where CP impacts virus biology. Instead, selective examples will be used to illustrate these regulatory activities.

CP regulation of viral translation

Unlike viruses with double-strand (ds) or negative(-)-strand RNA genomes, the vast majority of (+)-strand RNA viruses do not encapsidate replication proteins. Thus, the viral genome must be translated before replication can ensue. As one of the few, or possibly the only viral protein present before the initiation of translation, CPs are likely candidates for having regulatory roles in the translation of viral proteins. The regulatory roles of CPs involve interaction with the viral RNA sequences that direct translation as well as with the cellular translational factors, (Kapp and Lorsch, 2004). Examples selected based on the mechanisms of action are presented below.

Regulation through CP-RNA binding

The classic example for CP regulating translation comes from bacteriophage MS2. The MS2 CP dimer binds with low nanomolar affinity to an RNA hairpin motif that contains the initiation codon of the viral RNA dependent RNA polymerase (RdRp) and inhibits translation of the RdRp (Bernardi and Spahr, 1972; Carey et al., 1983; Lodish and Zinder, 1966). The structure of the CP-RNA complex exhibits sequence-specific recognition (Valegård et al., 1994). Interestingly, the motif regulated by the MS2 CP also functions as an encapsidation signal during virus assembly (Beckett and Uhlenbeck, 1988). Several CP mutants defective for virion assembly suppressed the RdRp translation better than did the wild type, presumably through elevated concentrations of the CP dimers (Peabody and Ely, 1992). These findings illustrate an interdependence of the structural and regulatory functions of CP, where CP binding to a specific RNA motif coordinately regulates viral RNA replication and virion assembly.

Significant insights into CP function in translation have also been gained from plant-infecting RNA viruses. The CP of the bacilliform Alfalfa mosaic virus (AMV; *Bromoviridae*) provides another well-characterized example of translational regulation. Successful initiation of AMV infection requires the addition of CP to the inoculum containing the viral multipartite genome (Bol et al., 1971). The basic NTR of the CP binds to the conserved 3′ untranslated regions (UTR) present in the AMV RNAs and the binding is required to activate the genome for infection (Baer et al., 1994; Zuidema et al., 1983a, 1983b).

The CP of BMV regulates the translation of the multipartite viral genome in a concentration-dependent manner (Yi et al., 2009). At a high concentration, the BMV CP binds to an RNA element named the B box within the 5′ UTRs in two of the BMV genomic RNAs that encode RNA replication proteins to down-regulate translation. Interestingly, the CP does not regulate the genomic RNA that encodes the CP. This mode of regulation could ensure proper expression of viral replication proteins from the BMV genomic RNAs.

The Hepatitis C virus (HCV; *Flaviviridae*) core protein can specifically bind the internal ribosome entry site (IRES) in the 5′ UTR of the viral genome to suppress the translation of genes downstream (Boni et al., 2005; Shimoike et al., 1999). Positively-charged residues in the N-terminal portion of the HCV core protein are required for the translation repression (Li et al., 2003; Shimoike et al., 2006). HCV core protein does not act as a simple translation repressor, however, since it stimulated translation when expressed at lower concentrations (Boni et al., 2005; Lourenço et al., 2008).

Regulation through CP-protein interaction

The ability of viral CPs to interact with RNA elements may act in concert with cellular translation factors. The AMV CP was found to interact with the host translation initiation factors eIF4G and eIFiso4G that bind the 5′ cap structure of mRNA (Krab et al., 2005). Since the AMV CP also binds to the 3′ UTR of the viral RNAs, interaction with the translation factors that bind to the 5′ UTR will functionally circularize the capped viral RNAs. This could facilitate ribosome reloading in translation. Furthermore, the AMV CP is effectively mimicking the function of host PolyA-binding protein (PABP) (Neeleman et al., 2001). The CP of Rubella virus (*Togaviridae*) specifically binds and sequesters the host PABP (Ilkow et al., 2008). Sequestration of PABP suppresses both the translation of host mRNAs and the viral RNAs that contain 3′ polyA tails. Impairing viral RNA translation may promote the encapsidation of viral RNAs while suppressing translation of host mRNAs perhaps reduce host immune responses to viral infection (see sections below).

CP regulation of viral RNA synthesis

RNA viruses replicate in specialized environments formed from cellular membranes, viral replication factories (Den Boon et al., 2010). Current models suggest that viral replication proteins and viral RNAs are trafficked to these factories (Ahlquist, 2006). Although CPs of (+)-strand RNA viruses are not required to form the factories, viral genome encapsidation is functionally coupled to its replication for several viruses (Annamalai and Rao, 2006; Khromykh et al., 2001; Nugent et al., 1999; Venter et al., 2005). There is also growing evidence that CPs of multiple RNA viruses have an active role in viral RNA synthesis through binding of the viral RNA and/or interaction with viral RNA replication-associated proteins.

Regulating RNA synthesis by CP-RNA interactions

In addition to the effects on translation, binding to the 3′ UTR of viral RNAs by the AMV CP can regulate RNA synthesis. One model suggests that the interaction stimulates RNA synthesis by reorganizing the conformation of 3′ UTR to facilitate the recognition of the promoter for minus-strand synthesis by the viral RdRp (Guogas et al., 2004; Reichert et al., 2007). An alternative model proposes that the change in 3′ UTR conformation upon CP binding blocks RNA synthesis (Chen and Olsthoorn, 2010; Olsthoorn et al., 1999). A key to reconciling these discrepant observations may be that the CP strongly stimulated viral RNA replication at low concentrations, but inhibits replication at higher concentrations (Guogas et al., 2005). The stepwise binding of CP to multiple elements in the 3′ UTR of the AMV RNA may switch AMV RNAs from translation to replication to virion assembly (Petrillo et al., 2005; Reusken et al., 1994). The BMV CP has also been implicated in viral RNA replication through its recognition of the promoter for BMV minus-strand RNA synthesis when present in low amounts (Yi et al., 2009; Zhu et al., 2007). A single nucleotide change to the promoter that dramatically reduces BMV RNA replication (Sivakumaran et al., 1999; Kim and Kao, 2001) also decreases binding by the CP (Zhu et al., 2007).

The N proteins of the *Coronaviridae* have been extensively studied for their participation in viral RNA synthesis. Replicons

derived from SARS-CoV and Transmissible gastroenteritis coronavirus (TGEV) both required a functional N gene for efficient replication (Almazán et al., 2004; Schelle et al., 2005). Furthermore, antiserum specific to N inhibited Murine hepatitis virus (MHV) RNA synthesis in vitro (Compton et al., 1987). The N protein could increase transcription of the nested and 3′ co-terminal subgenomic mRNAs whose expression is under the control of transcription-regulating sequences (TRS) (Baric et al., 1988; Grossoehme et al., 2009; Keane et al., 2012; Zúñiga et al., 2010). The N-protein remodels the TRS by specific high-affinity binding through its N-terminal domain and nonspecific chaperoning activity of the disordered and positively charged linker region (Grossoehme et al., 2009; Keane et al., 2012).

Regulation of RNA synthesis through CP-replication protein interaction

Both the AMV CP and the SARS-CoV N protein can bind to the viral replication complex in addition to *cis*-acting to regulate viral RNA synthesis (Keane and Giedroc, 2013; Reichert et al., 2007; Verheije et al., 2010). The HCV core protein binds to a region of HCV RdRp that encompasses the catalytic site and inhibits RNA synthesis in vitro (Kang et al., 2009). Exogenous expression of the core protein in hepatocytes suppressed the replication of the HCV subgenomic replicon that lacks the core-coding sequence (Kang et al., 2009). Furthermore, the HCV full-length replicon also accumulated less efficiently than the subgenomic replicon in cells (Pietschmann et al., 2002).

The CP of Rubella virus binds to the viral replication-associated protein, P150, and regulates the replication of Rubella virus replicon (Tzeng et al., 2006). The expression of CP particularly rescued the replication of a replicon carrying a lethal deletion of an arginine-rich motif in P150 (Tzeng and Frey, 2003; Tzeng et al., 2006). Introduction of the CP gene into the deleted region of the P150 gene yielded a viable subgenomic replicon that accumulated with wild-type kinetics (Tzeng and Frey, 2009). A cluster of positively-charged residues close to the RNA-binding region in the NTA of the CP was necessary for the complementation. This finding is unique in that the CP shares a common function with part of the replication protein, likely RNA binding, for viral RNA synthesis. Furthermore, the Rubella virus CP enhanced the replication of subgenomic replicons at low CP levels, but inhibited replication at high CP levels (Chen and Icenogle, 2004; Tzeng and Frey, 2005). Whether the effects are due to interaction with the replication proteins or specific recognition of the virus RNA remains to be determined.

Members of the *Caliciviridae* provide additional examples of CP regulating RdRp activities. A single amino acid mutation in the P domain of capsid protein VP1 of Murine norovirus (MNV: *Caliciviridae*) attenuated pathogenicity in mice as well as decreased MNV replication (Bailey et al., 2008; Strong et al., 2012). A MNV replicon with a frameshift mutation that disrupted VP1 expression was defective for RNA replication, and expression of VP1 *in trans* rescued its replication (Subba-Reddy et al., 2012). Co-expression of the VP1 and RdRp of the genogroup II.4 human norovirus also significantly enhanced RNA synthesis by the viral RdRp (Ranjith-Kumar et al., 2011; Subba-Reddy et al., 2012). In this assay, the S domain of VP1 was necessary and sufficient to increase viral RNA synthesis.

CP regulation of host innate immune responses

Innate immune responses can rapidly active mechanisms to limit viral infection. The innate immune responses are activated by receptors that recognize patterns in pathogen molecules. In vertebrates, the activation of these receptors can lead to interferon (IFN) production. IFN then activates the JAK-STAT pathway, inducing a suite of antiviral effectors (Versteeg and García-Sastre, 2010). Viral CPs, along with a suite of other viral proteins, can interfere with the signaling leading to IFN production and action (Versteeg and García-Sastre, 2010).

CP regulation of host immune responses is generally mediated through their interaction with host proteins. In some cases, RNA-binding by the CP, especially in the plant RNA silencing responses, can inhibit the host responses.

CP regulation of host responses through CP-protein interactions

Perhaps no other (+)-strand virus has received more attention than HCV with regard to the innate immune responses. The HCV core protein interferes with multiple components of the signaling cascades that lead to IFN production (Arnaud et al., 2010; Bode et al., 2003; Ciccaglione et al., 2007; de Lucas et al., 2005; Garaigorta and Chisari, 2009; Heim et al., 1999; Lin et al., 2006; Oshiumi et al., 2010). The ability of HCV core protein to interact multiple components of the innate immune pathway is likely related to core protein possessing regions characterized by intrinsic disorder. However, most of the IFN antagonizing mechanisms proposed for core protein are based on ectopically-expressed proteins or subgenomic replicons, which recapitulates only some aspects of the virus infection cycle.

The N protein, among several other viral proteins of the SARS-CoV, can interfere with IFN production. The N protein inhibits the activation of transcription factors IRF-3 and NF-κB that control the transcription of IFN genes (Kopecky-Bromberg et al., 2007; Totura and Baric, 2012). This activity may require the interaction of N protein with RNA since the truncated N protein impaired for RNA binding was debilitated for the ability to antagonize signal transduction by IFN (Lu et al., 2011).

Alphaviruses suppresses IFN production through the general down-regulation of host gene expression (Garmashova et al., 2007). The CPs of the Venezuelan and Eastern equine encephalitis viruses (VEEV and EEEV) interfere with nucleocytoplasmic trafficking of host mRNAs to decrease cellular gene expression (Garmashova et al., 2007). This activity is mediated through the interaction between the flexible N-terminal half of the CP and the nuclear pore complex (Atasheva et al., 2008, 2010). The EEEV CP also upregulated phosphorylation of the translation initiation factor eIF-2a to block the capped mRNA translation (Aguilar et al., 2007). Similar up-regulation of eIF-2a phosphorylation has also been suggested for the CP of Semliki forest virus (Favre et al., 1996).

Plant innate immune receptors are structurally and functionally similar to their counterparts in animals (Greeff et al., 2012; Schwessinger and Ronald, 2012). Therefore, it is not surprising that the plant viral CP can also act in innate immune responses. The plant Resistance (R) genes, which convey innate immune response, have been proposed to act either directly through interactions with the pathogen molecules, or indirectly by guarding cellular proteins that are either required for pathogen infection or can respond to infection (Knepper and Day, 2010).

R genes products interact with the CPs in several viruses, including Potato virus X (PVX; *Alphaflexiviridae*), Cucumber mosaic virus (CMV; *Bromoviridae*), and Turnip crinkle virus (TCV: *Tombusviridae*) (Kang et al., 2005). The most extensively characterized is TCV, where a dominant gene HRT (*Hypersensitive Response to TCV*) confers hypersensitive response (HR) in the inoculated leaves to limit TCV spread. A recessive gene, RRT (*Regulator of Resistance to TCV*), also contributes to resistance (Cooley et al., 2000; Kachroo et al., 2000). Overexpression of the CP in seedlings wild-type for HRT resulted in HR-induced necrosis (Cooley et al., 2000). A

transcription factor TIP (TCV-interacting protein) in the NAC family of developmental regulators in Arabidopsis has been identified to bind to the arginine-rich NTA of the TCV CP in the yeast two-hybrid assay and in vitro (Ren et al., 2000, 2005). The link between TIP and the R genes is unclear since insertion-inactivation of TIP did not affect the HR response to TCV infection, although an increase in CMV infection was observed (Jeong et al., 2008).

CP and apoptosis

Apoptosis plays an important role in the vertebrate immune system against virus (Barber, 2001). It regulates the development of immune system cells as well as eliminates the infected cells (Roulston et al., 1999). There are two pathways to activate apoptosis (Elmore, 2007). The extrinsic pathway initiates with recognition of cytokines such as tumor necrosis factor (TNF) by transmembrane receptors that leads to the activation of effector caspases. The intrinsic pathway is initiated by a diverse array of intracellular signals that act on sensors such as p53 and the signals are propagated to the mitochondria. A loss of mitochondrial transmembrane potential activates caspases. Not surprisingly, some viruses can avoid the elicitation of apoptosis. However, others viruses can stimulate apoptosis as part of their pathogenesis or dissemination (Everett and McFadden, 1999). Therefore, controlling the apoptosis machinery is crucial to the viral infection cycle. Numerous viral factors, including the viral CP, modulate apoptosis (Galluzzi et al., 2008).

The HCV core protein affects apoptosis in many different ways (Jahan et al., 2012). Particularly, it exhibits both pro- and anti-apoptotic properties, such as its ability to stimulate or suppress p53 or TNF-mediated apoptosis (Chou et al., 2005; Marusawa et al., 1999; Otsuka et al., 2000; Ray et al., 1998). The apparently contradictory functions may be linked to either lytic (activating apoptosis) or chronic (suppressing apoptosis) HCV infection (Guicciardi and Gores, 2005). Multiple regions in core protein have been mapped to interfere with different apoptotic pathways (Cao et al., 2004; Kang et al., 2009; Mohd-Ismail et al., 2009; Moorman et al., 2003). However, the relative contributions of these factors to HCV infection remains to be better characterized since they will depend on the expression level of core protein, the cell type, and the apoptotic stimuli.

The core protein of WNV exhibits both suppressive and stimulatory effects on apoptosis. Expression of WNV core protein suppresses apoptosis through activation of the phosphatidylinositol 3 kinase (PI3K) and its downstream target, Akt, a pro-survival kinase (Urbanowski and Hobman, 2013). In contrast, the precursor WNV core protein with the C-terminal signal peptide induced p53-mediated apoptosis (Yang et al., 2008). The precursor core protein promoted abnormal p53 accumulation by sequestering the HDM2 ubiquitin ligase that targets p53 for degradation. An explanation for the opposing effects is that the release of core protein following virus entry inhibits apoptosis in early stages of infection and the accumulation of the precursor core protein triggers apoptosis later in the infection cycle (Lee et al., 2005).

The CP of Rubella virus can confer resistance to apoptosis in infected cells by localizing to the mitochondria and disrupting the function of Bax, a mitochondrial pro-apoptotic protein (Ilkow et al., 2011). Importantly, recombinant Rubella virus with mutant CP that failed to block apoptosis is defective for replication, implying that the anti-apoptotic activity of CP is essential for Rubella virus propagation.

Several members of the *Picornaviridae* have CPs with pro-apoptotic activity. Expressing recombinant VP1 capsid protein of the Foot-and-mouth disease virus activated apoptosis through down-regulation of the Akt pro-survival signaling pathway (Peng et al., 2004). The VP3 of avian encephalomyelitis virus was localized to mitochondria and induced apoptosis (Liu et al., 2002). Lastly, a single amino acid substitution in the VP1 capsid protein of Coxsackie virus B2 (CVB2) transformed a noncytolytic virus to a cytolytic one, causing apoptotic responses that included caspase activation and DNA fragmentation (Gullberg et al., 2010).

CP affecting host immune responses through RNA binding

Viruses that infect plants, invertebrates and fungi tend to target the RNA interference (RNAi)-based pathways to overcome the host immune responses (Ding, 2010). The RNAi pathway uses the endoribonuclease Dicer to cleave the double-strand viral RNA replication intermediates. The dsRNA fragments can be unwound to form the siRNA that can guide the RNA silencing initiator complex (RISC) to degrade viral RNA through recognition of complementary sequences. Plant viruses are known to encode a diverse set of viral suppressors of RNA silencing (Burgyán and Havelda, 2011). The CP of the TCV is a suppressor of local and systemic RNA silencing (Qu et al., 2003). The TCV CP may sequester siRNAs from assembly into RISC complexes through the N-terminal RNA binding region (Mérai et al., 2006; Thomas et al., 2003). The TCV CP additionally interacts with the Argonaute protein 1 (AGO1) to disrupt RISC complex formation and to impact the availability of Dicer through an AGO1-dependent homeostatic network (Azevedo et al., 2010; Deleris et al., 2006).

Other activities of the CP

Several additional encapsidation-independent activities of viral CPs deserve mention because of their importance to viral infection and pathogenesis. Plant viruses move from cell to cell through the plasmodesmatas and systemically through the plant's vasculature. These processes are mediated by the viral movement proteins (MPs), but CPs of some viruses have also been demonstrated to play a role. Mutation of a single residue on the CP of Carnation ringspot virus (CRSV: *Tombusviridae*) abolished virus systemic infection without disrupting the encapsidation function of the CP (Sit et al., 2001). A similar effect was observed in the related Red clover necrotic mosaic virus (*Tombusviridae*) where virus with mutations of the CP could systemically infect *N. benthamiana* only at a low temperature (Xiong et al., 1993). Deletions in the CP of Cowpea chlorotic mosaic virus and Cucumber mosaic virus of the *Bromoviridae* prevented virion formation yet supported systemic or local spread of viral RNAs (Kaplan et al., 1998; Schmitz and Rao, 1998; Schneider et al., 1997). Specific interactions between the MP and CP and/or between CP and host factors are likely to be required for these activities.

(+)-strand RNA viruses replicate and assemble in the cytoplasm. However, some viruses have a significant proportion of their CP localized to the nucleus (Hiscox, 2003,2007). Nuclear-localized N protein of SARS-CoV and VP1 capsid protein of Coxsackie virus B3 (*Picornaviridae*) have been demonstrated to induce deregulation of the cell cycle (Surjit et al., 2006; Wang et al., 2012). For Dengue virus (DENV, *Flaviviridae*), the core protein acts as histone mimics to disrupt nucleosome formation (Colpitts et al., 2011), and the nuclear localization of the DENV core protein is required to sensitize cells to Fas-induced apoptosis (Limjindaporn et al., 2007; Netsawang et al., 2010). The nuclear localization of CP of Japanese encephalitis virus (*Flaviviridae*) and the N protein of Porcine reproductive and respiratory syndrome virus (*Coronaviridae*) are linked to virus titer and pathogenesis (Lee et al., 2006; Mori et al., 2005). The diverse functions of the nuclear localized CP highlight how RNA viruses target the nucleus to sequester resources for virus replication and to disrupt host responses.

Summary and perspectives

The past decade has seen a significant expansion in our understanding of the regulatory functions of CPs in virus infection cycles and host responses. The CPs of multiple (+)-strand RNA viruses can regulate viral genome translation and replication and host cell immune responses. In several cases, the CPs exert both stimulatory and inhibitory effects on the same viral infection processes or host responses. This likely reflects that the CP oligomerization is a function of subunit concentration. The state of oligomerization could change the interaction interface of CPs and the kinetics of binding to RNAs or proteins. This could, in turn, influence interpretation of results from experiments, especially where the CP was expressed ectopically rather than in the context of virus infection.

Finally, it is important to note that the regulatory activities of the CPs of (+)-strand RNA viruses mirror those of nucleocapsid proteins of (-)-strand and dsRNA viruses (Tao and Ye, 2010). (-)-strand and dsRNA viruses replicate within virion core structures or require the presence of the nucleocapsid protein. The nucleocapsid protein has been shown to bridge the interaction between RNA template and viral replication proteins (Green and Luo, 2009), or allosterically regulate replication protein activities (Lawton et al., 1997; McClain et al., 2010; Newcomb et al., 2009). Therefore, some of the lessons already learned from viruses whose infection cycles are more intimately intertwined with the nucleocapsid proteins could be instructive to those who study (+)-strand RNA viruses. Furthermore, most depictions of RNA viruses inaccurately leave the capsid at the surface of the infected cell. This should be reconsidered, given that some of the CP likely interacts with the viral and host factors to contribute to the infection process.

Acknowledgments

We thank Laura Kao for editing and members of the IU and TAMU Cereal Killers, former and present, for wonderful discussions through the years and for being willing to do Friday afternoon experiments. This work was supported by the NIH Grant 1R01AI090280 to C.K.

References

Abad-Zapatero, C., Abdel-Meguid, S.S., Johnson, J.E., Leslie, A.G., Rayment, I., Rossmann, M.G., Suck, D., Tsukihara, T., 1980. Structure of southern bean mosaic virus at 2.8 A resolution. Nature 286, 33–39.

Aguilar, P.V, Weaver, S.C., Basler, C.F., 2007. Capsid protein of eastern equine encephalitis virus inhibits host cell gene expression. J. Virol. 81, 3866–3876.

Ahlquist, P., 2006. Parallels among positive-strand RNA viruses, reverse-transcribing viruses and double-stranded RNA viruses. Nat. Rev. Microbiol. 4, 371–382.

Almazán, F., Galán, C., Enjuanes, L., Almaza, F., Gala, C., 2004. The nucleoprotein is required for efficient coronavirus genome replication. J. Virol. 78, 12638–12688.

Annamalai, P., Rao, A.L.N, 2006. Packaging of brome mosaic virus subgenomic RNA is functionally coupled to replication-dependent transcription and translation of coat protein. J. Virol. 80, 10096–10108.

Arnaud, N., Dabo, S., Maillard, P., Budkowska, A., Kalliampakou, K.I., Mavromara, P., Garcin, D., Hugon, J., Gatignol, A., Akazawa, D., et al., 2010. Hepatitis C virus controls interferon production through PKR activation. PloS One 5, e10575.

Atasheva, S., Garmashova, N., Frolov, I., Frolova, E., 2008. Venezuelan equine encephalitis virus capsid protein inhibits nuclear import in mammalian but not in mosquito cells. J. Virol. 82, 4028–4041.

Atasheva, S., Fish, A., Fornerod, M., Frolova, E.I., 2010. Venezuelan equine Encephalitis virus capsid protein forms a tetrameric complex with CRM1 and importin alpha/beta that obstructs nuclear pore complex function. J. Virol. 84, 4158–4171.

Azevedo, J., Garcia, D., Pontier, D., Ohnesorge, S., Yu, A., Garcia, S., Braun, L., Bergdoll, M., Hakimi, M.A., Lagrange, T., et al., 2010. Argonaute quenching and global changes in Dicer homeostasis caused by a pathogen-encoded GW repeat protein. Genes Dev. 24, 904–915.

Baer, M., Houser, F., Loesch-Fries, L.S., Gehrke, L., 1994. Specific RNA binding by amino-terminal peptides of alfalfa mosaic virus coat protein. EMBO J. 13, 727–735.

Bailey, D., Thackray, L.B., Goodfellow, I.G., 2008. A single amino acid substitution in the murine norovirus capsid protein is sufficient for attenuation in vivo. J. Virol. 82, 7725–7728.

Barber, G.N., 2001. Host defense, viruses and apoptosis. Cell Death Differ. 8, 113–126.

Baric, R.S., Nelson, G.W., Fleming, J.O., Deans, R.J., Keck, J.G., Casteel, N., Stohlman, S. A., 1988. Interactions between coronavirus nucleocapsid protein and viral RNAs: implications for viral transcription. J. Virol. 62, 4280–4287.

Beckett, D., Uhlenbeck, O.C., 1988. Ribonucleoprotein complexes of R17 coat protein and a translational operator analog. J. Mol. Biol. 204, 927–938.

Bernardi, A, Spahr, P.F, 1972. Nucleotide sequence at the binding site for coat protein on RNA of bacteriophage R17. Proc. Nat. Acad. Sci. USA 69, 3033–3037.

Bertolotti-ciarlet, A., White, L.J., Chen, R., Prasad, V., Estes, M.K., Prasad, B.V.V., 2002. Structural requirements for the assembly of norwalk virus-like particles. J. Virol. 76, 4044–4055.

Bode, J.G., Ludwig, S., Ehrhardt, C., Albrecht, U., Erhardt, A., Schaper, F., Heinrich, P. C., Häussinger, D., 2003. IFN-alpha antagonistic activity of HCV core protein involves induction of suppressor of cytokine signaling-3. FASEB J. 17, 488–490.

Bol, J.F., 2008. Role of capsid proteins. Methods Mol. Biol. (Clifton, N.J.) 451, 21–31.

Bol, J., Vloten-Doting, L.Van, Jaspars, E., 1971. A functional equivalence of top component a RNA and coat protein in the initiation of infection by Alfalfa Mosaic Virus. Virology 85, 73–85.

Boni, S., Lavergne, J.-P., Boulant, S., Cahour, A., 2005. Hepatitis C virus core protein acts as a trans-modulating factor on internal translation initiation of the viral RNA. J. Biol. Chem. 280, 17737–17748.

Bottcher, B., Crowther, R.A., 1996. Difference imaging reveals ordered regions of RNA in turnip yellow mosaic virus. Structure 4, 387–394.

Boulant, S., Vanbelle, C., Ebel, C., Penin, F., Lavergne, J.-P., 2005. Hepatitis C virus core protein is a dimeric alpha-helical protein exhibiting membrane protein features. J. Virol. 79, 11353–11365.

Burgyán, J., Havelda, Z., 2011. Viral suppressors of RNA silencing. Trends Plant Sci. 16, 265–272.

Callaway, A., Gillock, E.T., Sit, T.L., Lommel, S.A., 2001. The multifunctional capsid proteins of plant RNA viruses. Ann. Rev. Phytopathol. 39, 419–460.

Cao, Y., Hamada, T., Matsui, T., Date, T., Iwabuchi, K., 2004. Hepatitis C virus core protein interacts with p53-binding protein, 53BP2/Bbp/ASPP2, and inhibits p53-mediated apoptosis. Biochem. Biophys. Res. Commun. 315, 788–795.

Carey, J., Cameron, V., De Haseth, P.L., Uhlenbeck, O.C., 1983. Sequence-specific interaction of R17 coat protein with its ribonucleic acid binding site. Biochemistry 22, 2601–2610.

Chang, C.-K., Hsu, Y.-L., Chang, Y.-H., Chao, F.-A., Wu, M.-C., Huang, Y.-S., Hu, C.-K., Huang, T.-H., 2009. Multiple nucleic acid binding sites and intrinsic disorder of severe acute respiratory syndrome coronavirus nucleocapsid protein: implications for ribonucleocapsid protein packaging. J. Virol. 83, 2255–2264.

Chen, M., Icenogle, J., 2004. Rubella virus capsid protein modulates viral genome replication and virus infectivity. J. Virol. 78, 4314–4322.

Chen, S.-C., Olsthoorn, R.C.L., 2010. In vitro and in vivo studies of the RNA conformational switch in Alfalfa mosaic virus. J. Virol. 84, 1423–1429.

Chen, C.-Y., Chang, C.-K., Chang, Y.-W., Sue, S.-C., Bai, H.-I., Riang, L., Hsiao, C.-D., Huang, T.-H., 2007. Structure of the SARS coronavirus nucleocapsid protein RNA-binding dimerization domain suggests a mechanism for helical packaging of viral RNA. J. Mol. Biol. 368, 1075–1086.

Chen, R., Neill, J.D., Estes, M.K., Prasad, B.V.V., 2006. X-ray structure of a native calicivirus: structural insights into antigenic diversity and host specificity. Proc. Nat. Acad. Sci. USA 103, 8048–8053.

Chen, Z.G., Stauffacher, C., Li, Y., Schmidt, T., Bomu, W., Kamer, G., Shanks, M., Lomonossoff, G., Johnson, J.E., 1989. Protein–RNA interactions in an icosahedral virus at 3.0 A resolution. Science 245, 154–159.

Choi, H., Tong, L., Minor, W., Dumas, P., Boege, U., Rossmann, M.G., Gerd, W., 1991. Structure of Sindbis virus core protein reveals a chymotrypsin-like serine proteinase and the organization of the virion. Nature 354, 37–43.

Chou, A.-H., Tsai, H.-F., Wu, Y.-Y., Hu, C.-Y., Hwang, L.-H., Hsu, P.-I., Hsu, P.-N., 2005. Hepatitis C virus core protein modulates TRAIL-mediated apoptosis by enhancing Bid cleavage and activation of mitochondria apoptosis signaling pathway. J. Immunol. 174, 2160–2166.

Ciccaglione, A.R., Stellacci, E., Marcantonio, C., Muto, V., Equestre, M., Marsili, G., Rapicetta, M., Battistini, A., 2007. Repression of interferon regulatory factor 1 by hepatitis C virus core protein results in inhibition of antiviral and immunomodulatory genes. J. Virol. 81, 202–214.

Colpitts, T.M., Barthel, S., Wang, P., Fikrig, E., 2011. Dengue virus capsid protein binds core histones and inhibits nucleosome formation in human liver cells. PloS One 6, e24365.

Compton, S.R., Rogers, D.B., Holmes, K.V., Fertsch, D., Remenick, J., Mcgowant, J.J., 1987. In vitro replication of mouse hepatitis virus strain A59. J. Virol. 61, 1814–1820.

Cooley, M.B., Pathirana, S., Wu, H.J., Kachroo, P., Klessig, D.F., 2000. Members of the Arabidopsis HRT/RPP8 family of resistance genes confer resistance to both viral and oomycete pathogens. Plant Cell 12, 663–676.

Deleris, A., Gallego-Bartolome, J., Bao, J., Kasschau, K.D., Carrington, J.C., Voinnet, O., 2006. Hierarchical action and inhibition of plant Dicer-like proteins in antiviral defense. Science 313, 68–71.

Den Boon, J.A., Diaz, A., Ahlquist, P., 2010. Cytoplasmic viral replication complexes. Cell Host Microbe 8, 77–85.

de Lucas, S., Bartolome, J., Carreno, V., 2005. Hepatitis C virus core protein down-regulates transcription of interferon-induced antiviral genes. J. Infect. Dis. 191, 93–99.
Ding, S.-W., 2010. RNA-based antiviral immunity. Nat. Rev. Immunol. 10, 632–644.
Dokland, T., Walsh, M., Mackenzie, J.M., Khromykh, A.A, Ee, K.-H., Wang, S., 2004. West Nile virus core protein; tetramer structure and ribbon formation. Structure 12, 1157–1163.
Donaldson, E.F., Lindesmith, L.C., Lobue, A.D., Baric, R.S., 2010. Viral shape-shifting: norovirus evasion of the human immune system. Nat. Rev. Microbiol. 8, 231–241.
Dunker, A.K., Cortese, M.S., Romero, P., Iakoucheva, L.M., Uversky, V.N., 2005. Flexible nets. The roles of intrinsic disorder in protein interaction networks. FEBS J. 272, 5129–5148.
Dunker, A.K., Silman, I., Uversky, V.N., Sussman, J.L., 2008. Function and structure of inherently disordered proteins. Curr. Opin. Struct. Biol, 18, 756–764.
Elmore, S., 2007. Apoptosis: a review of programmed cell death. Toxicol. Pathol. 35, 495–516.
Everett, H., McFadden, G., 1999. Apoptosis: an innate immune response to virus infection. Trends Microbiol. 7, 160–165.
Favre, D., Studer, E., Michel, M.R., 1996. Semliki Forest virus capsid protein inhibits the initiation of translation by upregulating the double-stranded RNA-activated protein kinase (PKR). Biosci. Rep. 16, 485–511.
Fisher, A., Johnson, J., 1993. Ordered duplex RNA controls capsid archtecture in an icosahedral animal virus. Nature 361, 176–179.
Galluzzi, L., Brenner, C., Morselli, E., Touat, Z., Kroemer, G., 2008. Viral control of mitochondrial apoptosis. PLoS Path. 4, e1000018.
Garaigorta, U., Chisari, F.V., 2009. Hepatitis C virus blocks interferon effector function by inducing protein kinase R phosphorylation. Cell Host Microbe. 6, 513–522.
Garmashova, N., Atasheva, S., Kang, W., Weaver, S.C., Frolova, E., Frolov, I., 2007. Analysis of Venezuelan equine encephalitis virus capsid protein function in the inhibition of cellular transcription. J. Virol. 81, 13552–13565.
Greeff, C., Roux, M., Mundy, J., Petersen, M., 2012. Receptor-like kinase complexes in plant innate immunity. Front. Plant Sci. 3, 209.
Green, T.J., Luo, M., 2009. Structure of the vesicular stomatitis virus nucleocapsid in complex with the nucleocapsid-binding domain of the small polymerase cofactor. Proc. Nat. Acad. Sci. USA 106, 11713–11718.
Grossoehme, N.E., Li, L., Keane, S.C., Liu, P., Dann, C.E., Leibowitz, J.L., Giedroc, D.P., 2009. Coronavirus N protein N-terminal domain (NTD) specifically binds the transcriptional regulatory sequence (TRS) and melts TRS-cTRS RNA duplexes. J. Mol. Biol. 394, 544–557.
Guicciardi, M.E., Gores, G.J., 2005. Apoptosis: a mechanism of acute and chronic liver injury. Gut 54, 1024–1033.
Gullberg, M., Tolf, C., Jonsson, N., Polacek, C., Precechtelova, J., Badurova, M., Sojka, M., Mohlin, C., Israelsson, S., Johansson, K., et al., 2010. A single coxsackievirus B2 capsid residue controls cytolysis and apoptosis in rhabdomyosarcoma cells. J. Virol. 84, 5868–5879.
Guogas, L.M., Filman, D.J., Hogle, J.M., Gehrke, L., 2004. Cofolding organizes alfalfa mosaic virus RNA and coat protein for replication. Science 306, 2108–2111.
Guogas, L.M., Laforest, S.M., Gehrke, L., 2005. Coat protein activation of alfalfa mosaic virus replication is concentration dependent. J. Virol. 79, 5752–5761.
Harrison, S.C., Olson, A.J., Schutt, C.E., Winkler, F.K., 1978. Tomato bushy stunt virus at 2.9 A resolution. Nature 276, 368–375.
Heim, M.H., Moradpour, D., Blum, H.E., 1999. Expression of Hepatitis C virus proteins inhibits signal transduction through the Jak-STAT Pathway. J. Virol. 73, 8469–8475.
Hiscox, J.A., 2003. The interaction of animal cytoplasmic RNA viruses with the nucleus to facilitate replication. Virus Res. 95, 13–22.
Hiscox, J.A., 2007. RNA viruses: hijacking the dynamic nucleolus. Nat. Rev. Microbiol. 5, 119–127.
Huang, Q., Yu, L., Petros, A.M., Gunasekera, A., Liu, Z., Xu, N., Hajduk, P., Mack, J., Fesik, S.W., Olejniczak, E.T., 2004. Structure of the N-terminal RNA-binding domain of the SARS CoV nucleocapsid protein. Biochemistry 43, 6059–6063.
Ilkow, C.S., Mancinelli, V., Beatch, M.D., Hobman, T.C., 2008. Rubella virus capsid protein interacts with poly(a)-binding protein and inhibits translation. J. Virol. 82, 4284–4294.
Ilkow, C.S., Goping, I.S., Hobman, T.C., 2011. The Rubella virus capsid is an anti-apoptotic protein that attenuates the pore-forming ability of Bax. PLoS Path. 7, e1001291.
Ivanyi-Nagy, R., Lavergne, J.-P., Gabus, C., Ficheux, D., Darlix, J.-L., 2008. RNA chaperoning and intrinsic disorder in the core proteins of Flaviviridae. Nucleic Acids Res. 36, 712–725.
Jahan, S., Ashfaq, U.A., Khaliq, S., Samreen, B., Afzal, N., 2012. Dual behavior of HCV Core gene in regulation of apoptosis is important in progression of HCC. Infect. Genet. Evol. 12, 236–239.
Jeong, R.-D., Chandra-Shekara, A.C., Kachroo, A., Klessig, D.F., Kachroo, P., 2008. HRT-mediated hypersensitive response and resistance to Turnip crinkle virus in Arabidopsis does not require the function of TIP, the presumed guardee protein. Mol. Plant-microbe Interact.: MPMI 21, 1316–1324.
Kachroo, P., Yoshioka, K., Shah, J., Dooner, H.K., Klessig, D.F., 2000. Resistance to turnip crinkle virus in Arabidopsis is regulated by two host genes and is salicylic acid dependent but NPR1, ethylene, and jasmonate independent. Plant Cell 12, 677–690.
Kakani, K., Reade, R., Rochon, D., 2004. Evidence that vector transmission of a plant virus requires conformational change in virus particles. J. Mol. Biol. 338, 507–517.
Kakani, K., Reade, R., Katpally, U., Smith, T., Rochon, D., 2008. Induction of particle polymorphism by cucumber necrosis virus coat protein mutants in vivo. J. Virol. 82, 1547–1557.
Kang, B.-C., Yeam, I., Jahn, M.M., 2005. Genetics of plant virus resistance. Ann. Rev. Phytopath. 43, 581–621.
Kang, S.-M., Choi, J.-K., Kim, S.-J., Kim, J.-H., Ahn, D.-G., Oh, J.-W., 2009. Regulation of hepatitis C virus replication by the core protein through its interaction with viral RNA polymerase. Biochem. Biophys. Res. Commun. 386, 55–59.
Kaplan, I.B., Zhang, L., Palukaitis, P., 1998. Characterization of Cucumber Mosaic Virus. Virology 246, 221–231.
Kapp, L.D., Lorsch, J.R., 2004. The molecular mechanics of eukaryotic translation. Ann. Rev. Biochem. 73, 657–704.
Katpally, U., Voss, N.R., Cavazza, T., Taube, S., Rubin, J.R., Young, V.L., Stuckey, J., Ward, V.K., Virgin, H.W., Wobus, C.E., et al., 2010. High-resolution cryo-electron microscopy structures of murine norovirus 1 and rabbit hemorrhagic disease virus reveal marked flexibility in the receptor binding domains. J. Virol. 84, 5836–5841.
Keane, S.C., Giedroc, D.P., 2013. Solution structure of mouse hepatitis virus (MHV) nsp3a and determinants of the interaction with MHV nucleocapsid (N) protein. J. Virol. 87, 3502–3515.
Keane, S.C., Liu, P., Leibowitz, J.L., Giedroc, D.P., 2012. Functional transcriptional regulatory sequence (TRS) RNA binding and helix destabilizing determinants of murine hepatitis virus (MHV) nucleocapsid (N) protein. J. Biol. Chem. 287, 7063–7073.
Khromykh, A.A., Varnavski, A.N., Petra, L., Westaway, E.G., Sedlak, P.L., 2001. Coupling between replication and packaging of Flavivirus RNA: evidence derived from the use of DNA-based full-length cDNA clones of Kunjin Virus. J. Virol. 75, 4633–4640.
Kim, CH., Kao, C.C., 2001. A mutant viral RNA promoter with an altered conformation retains efficient recognition by a viral RNA replicase through a solution-exposed adenine. RNA 7, 1476–1485.
Knepper, C., Day, B., 2010. From perception to activation: the molecular-genetic and biochemical landscape of disease resistance signaling in plants. Arabidopsis Book/Am. Soc. Plant Biol. 8, e012.
Kopecky-Bromberg, S. a, Martínez-Sobrido, L., Frieman, M., Baric, R.A., Palese, P., 2007. Severe acute respiratory syndrome coronavirus open reading frame (ORF) 3b, ORF 6, and nucleocapsid proteins function as interferon antagonists. J. Virol. 81, 548–557.
Krab, I.M., Caldwell, C., Gallie, D.R., Bol, J.F., 2005. Coat protein enhances translational efficiency of Alfalfa mosaic virus RNAs and interacts with the eIF4G component of initiation factor eIF4F. J. Gen. Virol. 86, 1841–1849.
Lawton, J.A., Zeng, C.Q., Mukherjee, S.K., Cohen, J., Estes, M.K., Prasad, B.V., 1997. Three-dimensional structural analysis of recombinant rotavirus-like particles with intact and amino-terminal-deleted VP2: implications for the architecture of the VP2 capsid layer. J. Virol. 71, 7353–7360.
Lee, C., Liao, C., Lin, Y., 2005. Flavivirus activates phosphatidylinositol 3-kinase signaling to block caspase-dependent apoptotic cell death at the early stage of virus infection. J. Virol. 79, 8388–8399.
Lee, C., Hodgins, D., Calvert, J.G., Welch, S.-K.W., Jolie, R., Yoo, D., 2006. Mutations within the nuclear localization signal of the porcine reproductive and respiratory syndrome virus nucleocapsid protein attenuate virus replication. Virology 346, 238–250.
Li, D., Takyar, S.T., Lott, W.B., Gowans, E.J., 2003. Amino acids 1–20 of the hepatitis C virus (HCV) core protein specifically inhibit HCV IRES-dependent translation in HepG2 cells, and inhibit both HCV IRES- and cap-dependent translation in HuH7 and CV-1 cells. J. Gen. Virol. 84, 815–825.
Liljas, L. 2011 Functional Role of Structural Disorder in Capsid Proteins, in: Uversky, V.N., Longhi, S., (Eds.). Flexible Viruses: Structural Disorder in Viral Proteins. John Wiley & Sons, Inc., Hoboken, NJ, USA.
Limjindaporn, T., Netsawang, J., Noisakran, S., Thiemmeca, S., Wongwiwat, W., Sudsaward, S., Avirutnan, P., Puttikhunt, C., Kasinrerk, W., Sriburi, R., et al., 2007. Sensitization to Fas-mediated apoptosis by dengue virus capsid protein. Biochem. Biophys. Res. Commun. 362, 334–339.
Lin, W., Kim, S.S., Yeung, E., Kamegaya, Y., Blackard, J.T., Kim, K.A., Holtzman, M.J., Chung, R.T., 2006. Hepatitis C virus core protein blocks interferon signaling by interaction with the STAT1 SH2 domain. J. Virol. 80, 9226–9235.
Liu, J., Wei, T., Kwang, J., 2002. Avian Encephalomyelitis Virus induces apoptosis via major structural protein VP3. Virology 300, 39–49.
Lodish, H., Zinder, N., 1966. Mutants of the bacteriophage f2 VIII. Control mechanism for phage-specific syntheses. J. Mol. Biol. 19, 333–348.
Lourenço, S., Costa, F., Débarges, B., Andrieu, T., Cahour, A., 2008. Hepatitis C virus internal ribosome entry site-mediated translation is stimulated by cis-acting RNA elements and trans-acting viral factors. FEBS J. 275, 4179–4197.
Lu, X., Pan, J., Tao, J., Guo, D., 2011. SARS-CoV nucleocapsid protein antagonizes IFN-β response by targeting initial step of IFN-β induction pathway, and its C-terminal region is critical for the antagonism. Virus Genes 42, 37–45.
Lucas, R.W., Larson, S.B., McPherson, A., 2002. The crystallographic structure of brome mosaic virus. J. Mol. Biol. 317, 95–108.
Marusawa, H., Hijikata, M., Chiba, T., Shimotohno, K., 1999. Hepatitis C virus core protein inhibits fas- and tumor necrosis factor alpha-mediated apoptosis via NF- κ B activation. J. Virol. 73, 4713–4721.
McClain, B., Settembre, E., Temple, B.R.S., Bellamy, A.R., Harrison, S.C., 2010. X-ray crystal structure of the rotavirus inner capsid particle at 3.8 A resolution. J. Mol. Biol. 397, 587–599.
Mérai, Z., Kerényi, Z., Kertész, S., Magna, M., Lakatos, L., Silhavy, D., 2006. Double-stranded RNA binding may be a general plant RNA viral strategy to suppress RNA silencing. J. Virol. 80, 5747–5756.

Mohd-Ismail, N.K., Deng, L., Sukumaran, S.K., Yu, V.C., Hotta, H., Tan, Y.-J., 2009. The hepatitis C virus core protein contains a BH3 domain that regulates apoptosis through specific interaction with human Mcl-1. J. Virol. 83, 9993–10006.
Moorman, J.P., Prayther, D., McVay, D., Hahn, Y.S., Hahn, C.S., 2003. The C-terminal region of hepatitis C core protein is required for Fas-ligand independent apoptosis in Jurkat cells by facilitating Fas oligomerization. Virology 312, 320–329.
Mori, Y., Okabayashi, T., Yamashita, T., Zhao, Z., Wakita, T., Yasui, K., Hasebe, F., Tadano, M., Konishi, E., Moriishi, K., et al., 2005. Nuclear localization of Japanese encephalitis virus core protein enhances viral replication. J. Virol. 79, 3448–3458.
Neeleman, L., Olsthoorn, R.C., Linthorst, H.J., Bol, J.F., 2001. Translation of a nonpolyadenylated viral RNA is enhanced by binding of viral coat protein or polyadenylation of the RNA. Proc. Nat. Acad. Sci. USA 98, 14286–14291.
Netsawang, J., Noisakran, S., Puttikhunt, C., Kasinrerk, W., Wongwiwat, W., Malasit, P., Yenchitsomanus, P., Limjindaporn, T., 2010. Nuclear localization of dengue virus capsid protein is required for DAXX interaction and apoptosis. Virus Res. 147, 275–283.
Newcomb, L.L., Kuo, R.-L., Ye, Q., Jiang, Y., Tao, Y.J., Krug, R.M., 2009. Interaction of the influenza a virus nucleocapsid protein with the viral RNA polymerase potentiates unprimed viral RNA replication. J. Virol. 83, 29–36.
Ni, P., Wang, Z., Ma, X., Das, N.C., Sokol, P., Chiu, W., Dragnea, B., Hagan, M., Kao, C.C., 2012. An examination of the electrostatic interactions between the N-terminal tail of the Brome Mosaic Virus coat protein and encapsidated RNAs. J. Mol. Biol. 419, 284–300.
Nugent, C.I., Johnson, K.L., Sarnow, P., Kirkegaard, K., 1999. Functional coupling between replication and packaging of poliovirus replicon RNA. J. Virol. 73, 427–435.
Olsthoorn, R.C., Mertens, S., Brederode, F.T., Bol, J.F., 1999. A conformational switch at the 3′ end of a plant virus RNA regulates viral replication. EMBO J. 18, 4856–4864.
Oshiumi, H., Ikeda, M., Matsumoto, M., Watanabe, A., Takeuchi, O., Akira, S., Kato, N., Shimotohno, K., Seya, T., 2010. Hepatitis C virus core protein abrogates the DDX3 function that enhances IPS-1-mediated IFN-beta induction. PloS One 5, e14258.
Otsuka, M., Kato, N., Lan, K., Yoshida, H., Kato, J., Goto, T., Shiratori, Y., Omata, M., 2000. Hepatitis C virus core protein enhances p53 function through augmentation of DNA binding affinity and transcriptional ability. J. Biol. Chem. 275, 34122–34130.
Peabody, D.S., Ely, K.R., 1992. Control of translational repression by protein–protein interactions. Nucleic Acids Res. 20, 1649–1655.
Peng, J.-M., Liang, S.-M., Liang, C.-M., 2004. VP1 of foot-and-mouth disease virus induces apoptosis via the Akt signaling pathway. J. Biol. Chem. 279, 52168–52174.
Petrillo, J.E., Rocheleau, G., Kelley-clarke, B., Gehrke, L., 2005. Evaluation of the conformational switch model for Alfalfa Mosaic Virus rna replication. J. Virol. 79, 5743–5751.
Pietschmann, T., Lohmann, V., Kaul, A., Rinck, G., Rutter, G., Strand, D., Bartenschlager, R., Krieger, N., 2002. Persistent and transient replication of full-length Hepatitis C Virus genomes in cell culture. J. Virol. 76, 4008–4021.
Prasad, B.V., Hardy, M.E., Dokland, T., Bella, J., Rossmann, M.G., Estes, M.K., 1999. X-ray crystallographic structure of the Norwalk virus capsid. Science 286, 287–290.
Qu, F., Ren, T., Morris, T., 2003. The coat protein of turnip crinkle virus suppresses posttranscriptional gene silencing at an early initiation step. J. Virol. 77, 511–522.
Ranjith-Kumar, C.T., Wen, Y., Baxter, N., Bhardwaj, K., Cheng Kao, C., 2011. A cell-based assay for RNA synthesis by the HCV polymerase reveals new insights on mechanism of polymerase inhibitors and modulation by NS5A. PloS One 6, e22575.
Ray, R.B., Meyer, K., Steele, R., Shrivastava, A., Aggarwal, B.B., Ray, R., 1998. Inhibition of tumor necrosis factor (TNF-alpha)-mediated apoptosis by hepatitis C virus core protein. J. Biol. Chem. 273, 2256–2259.
Reichert, V.L., Choi, M., Petrillo, J.E., Gehrke, L., 2007. Alfalfa mosaic virus coat protein bridges RNA and RNA-dependent RNA polymerase in vitro. Virology 364, 214–226.
Ren, T., Qu, F., Morris, T.J., 2000. HRT gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. Plant Cell 12, 1917–1926.
Ren, T., Qu, F., Morris, T.J., 2005. The nuclear localization of the Arabidopsis transcription factor TIP is blocked by its interaction with the coat protein of Turnip crinkle virus. Virology 331, 316–324.
Reusken, C.B., Neeleman, L., Bol, J.F., 1994. The 3′-untranslated region of alfalfa mosaic virus RNA 3 contains at least two independent binding sites for viral coat protein. Nucleic Acids Res. 22, 1346–1353.
Rossmann, M., Johnson, J., 1989. Icosahedral RNA virus structure. Ann. Rev. Biochem. 58, 533–573.
Rossmann, M., Arnold, E., Erickson, J.E., Frankenberger, E.A., Griffith, J.P., Hecht, H.-J., Johnson, J.E., Kamer, G., Luo, M., Mosser, A., et al., 1985. Structure of a human common cold virus and functional relationship to other picornaviruses. Nature 317, 145–153.
Roulston, A., Marcellus, R.C., Branton, P.E., 1999. Viruses and apoptosis. Ann. Rev. Microbiol. 53, 577–628.
Schelle, B., Karl, N., Ludewig, B., Siddell, S.G., 2005. Selective replication of Coronavirus Genomes that express nucleocapsid protein. J. Virol. 79, 6620–6630.
Schmitz, I., Rao, A., 1998. Deletions in the conserved amino-terminal basic arm of cucumber mosaic virus coat protein disrupt virion assembly but do not abolish infectivity and cell-to-cell movement. Virology 248, 323–331.
Schneider, W.L., Greene, A.E., Allison, R.F., 1997. The carboxy-terminal two-thirds of the cowpea chlorotic mottle bromovirus capsid protein is incapable of virion formation yet supports systemic movement. J. Virol 71, 4862–4865.
Schwessinger, B., Ronald, P.C, 2012. Plant innate immunity: perception of conserved microbial signatures. Ann. Rev. Plant Biol. 63, 451–482.
Shimoike, T., Mimori, S., Tani, H., Matsuura, Y., Miyamura, T., 1999. Interaction of Hepatitis C virus core protein with viral sense RNA and suppression of its translation. J. Virol. 73, 9718–9725.
Shimoike, T., Koyama, C., Murakami, K., Suzuki, R., Matsuura, Y., Miyamura, T., Suzuki, T., 2006. Down-regulation of the internal ribosome entry site (IRES)-mediated translation of the hepatitis C virus: critical role of binding of the stem-loop IIId domain of IRES and the viral core protein. Virology 345, 434–445.
Sit, T., Haikal, P., Callaway, A., Lommel, S., 2001. A single amino acid mutation in the Carnation Ringspot Virus capsid protein allows virion formation but prevents systemic infection. J. Virol 75, 9538–9542.
Sivakumaran, K., Kim, C.H., Tayon, R., Kao, C.C., 1999. RNA sequence and secondary structural determinants in a minimal viral promoter that directs replicase recognition and initiation of genomic plus-strand RNA synthesis. J. Mol. Biol. 294, 667–682.
Sorger, P.K., Stockley, P.G., Harrison, S.C., 1986. Structure and assembly of turnip crinkle virus. II. Mechanism of reassembly in vitro. J. Mol. Biol. 191, 639–658.
Speir, J.A., Bothner, B., Qu, C., Willits, D.A., Young, M.J., Johnson, J.E., 2006. Enhanced local symmetry interactions globally stabilize a mutant virus capsid that maintains infectivity and capsid dynamics. J. Virol. 80, 3582–3591.
Stockley, P.G., Rolfsson, O., Thompson, G.S., Basnak, G., Francese, S., Stonehouse, N.J., Homans, S.W., Ashcroft, A.E., 2007. A simple, RNA-mediated allosteric switch controls the pathway to formation of a T=3 viral capsid. J. Mol. Biol. 369, 541–552.
Strong, D.W., Thackray, L.B., Smith, T.J., Virgin, H.W., 2012. Protruding domain of capsid protein is necessary and sufficient to determine murine norovirus replication and pathogenesis in vivo. J. Virol. 86, 2950–2958.
Subba-Reddy, C.V., Yunus, M.A., Goodfellow, I.G., Kao, C.C., 2012. Norovirus RNA synthesis is modulated by an interaction between the viral RNA-dependent RNA polymerase and the major capsid protein, VP1. J. Virol. 86, 10138–10149.
Surjit, M., Liu, B., Chow, V.T.K., Lal, S.K., 2006. The nucleocapsid protein of severe acute respiratory syndrome-coronavirus inhibits the activity of cyclin–cyclin-dependent kinase complex and blocks S phase progression in mammalian cells. J. Biol. Chem. 281, 10669–10681.
Takeda, M., Chang, C., Ikeya, T., Güntert, P., Chang, Y., Hsu, Y., Huang, T., Kainosho, M., 2008. Solution structure of the c-terminal dimerization domain of SARS coronavirus nucleocapsid protein solved by the SAIL–NMR method. J. Mol. Biol. 380, 608–622.
Tao, Y.J., Ye, Q., 2010. RNA virus replication complexes. PLoS Path. 6, e1000943.
Thomas, C.L., Leh, V., Lederer, C., Maule, A.J., 2003. Turnip crinkle virus coat protein mediates suppression of RNA silencing in nicotiana benthamiana. Virology 306, 33–41.
Totura, A.L., Baric, R.S., 2012. SARS coronavirus pathogenesis: host innate immune responses and viral antagonism of interferon. Curr. Opin. Virol. 2, 264–275.
Tzeng, W., Frey, T., 2003. Complementation of a deletion in the Rubella Virus P150 nonstructural protein by the viral capsid protein. J. Virol. 77, 9502–9510.
Tzeng, W.-P., Frey, T.K., 2005. Rubella virus capsid protein modulation of viral genomic and subgenomic RNA synthesis. Virology 337, 327–334.
Tzeng, W.-P., Frey, T.K., 2009. Functional replacement of a domain in the rubella virus p150 replicase protein by the virus capsid protein. J. Virol. 83, 3549–3555.
Tzeng, W., Matthews, J., Frey, T., 2006. Analysis of rubella virus capsid protein-mediated enhancement of replicon replication and mutant rescue. J. Virol. 80, 3966–3974.
Urbanowski, M.D., Hobman, T.C., 2013. The West Nile virus capsid protein blocks apoptosis through a phosphatidylinositol 3-kinase-dependent mechanism. J. Virol. 87, 872–881.
Urbanowski, M.D., Ilkow, C.S., Hobman, T.C., 2008. Modulation of signaling pathways by RNA virus capsid proteins. Cell. Signal 20, 1227–1236.
Valegård, K., Liljas, L., Fridborg, K., Unge, T., 1990. The three-dimensional structural of the bacterial virus MS2. Nature 345, 36–41.
Valegård, K., Murray, J., Stockley, P., 1994. Crystal structure of an RNA bacteriophage coat protein-operator complex. Nature 371, 623–626.
Venter, P., Krishna, N., Schneemann, A., 2005. Capsid protein synthesis from replicating RNA directs specific packaging of the genome of a multipartite, positive-strand RNA virus. J. Virol. 79, 6239–6248.
Verheije, M.H., Hagemeijer, M.C., Ulasli, M., Reggiori, F., Rottier, P.J.M., Masters, P.S., De Haan, C.A.M., 2010. The coronavirus nucleocapsid protein is dynamically associated with the replication–transcription complexes. J. Virol. 84, 11575–11579.
Versteeg, G.A., García-Sastre, A., 2010. Viral tricks to grid-lock the type I interferon system. Curr. Opin. Microbiol. 13, 508–516.
Wang, T., Yu, B., Lin, L., Zhai, X., Han, Y., Qin, Y., Guo, Z., Wu, S., Zhong, X., Wang, Y., et al., 2012. A functional nuclear localization sequence in the VP1 capsid protein of coxsackievirus B3. Virology 433, 513–521.
Xiang, Y., Kakani, K., Reade, R., Hui, E., Rochon, D., 2006. A 38-amino-acid sequence encompassing the arm domain of the cucumber necrosis virus coat protein

functions as a chloroplast transit Peptide in infected plants. J. Virol 80, 7952–7964.
Xiong, Z., Kim, K., Giesman-Cookmeyer, D., Lommel, S., 1993. The roles of the red clover necrotic mosaic virus capsid and cell-to-cell movement proteins in systemic infection. Virology 192, 27–32.
Yang, M.-R., Lee, S.R., Oh, W., Lee, E.-W., Yeh, J.-Y., Nah, J.-J., Joo, Y.-S., Shin, J., Lee, H.-W., Pyo, S., et al., 2008. West Nile virus capsid protein induces p53-mediated apoptosis via the sequestration of HDM2 to the nucleolus. Cell. Microbiol. 10, 165–176.
Yi, G., Letteney, E., Kim, C.-H., Kao, C.C., 2009. Brome mosaic virus capsid protein regulates accumulation of viral replication proteins by binding to the replicase assembly RNA element. RNA 15, 615–626.
Yu, I.-M., Oldham, M.L., Zhang, J., Chen, J, 2006. Crystal structure of the severe acute respiratory syndrome (SARS) coronavirus nucleocapsid protein dimerization domain reveals evolutionary linkage between corona- and arteriviridae. J. Biol. Chem. 281, 17134–17139.
Zhu, J., Gopinath, K., Murali, A., Yi, G., Hayward, S.D., Zhu, H., Kao, C., 2007. RNA-binding proteins that inhibit RNA virus infection. Proc. Nat. Acad. Sci. USA 104, 3129–3134.
Zuidema, D., Bierhuizen, M.F., Jaspars, E.M., 1983a. Removal of the N-terminal part of alfalfa mosaic virus coat protein interferes with the specific binding to RNA 1 and genome activation. Virology 129, 255–260.
Zuidema, D., Bierhuizen, M., Cornelissen, B., 1983b. Coat protein binding sites on RNA 1 of alfalfa mosaic virus. Virology 369, 361–369.
Zúñiga, S., Cruz, J.L.G., Sola, I., Mateos-Gómez, P.A., Palacio, L., Enjuanes, L., 2010. Coronavirus nucleocapsid protein facilitates template switching and is required for efficient transcription. J. Virol. 84, 2169–2175.